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(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 2199 Addison Street, Berkeley, CA 94720 (US).

(72) Inventors: SZOKA, Francis, C. ; Hochstrasse 58, CH-8006 Zürich (CH). CHU, Chun-Jung ; 1544 8th Avenue, San Francisco, CA 94122 (US).

(74) Agents: MURASHIGE, Kate, H. et al.; Ciotti & Murashige, Irell & Manella, 545 Middlefield Road, Suite 200, Menlo Park, CA 94025 (US).

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(54) Title: LIPOSOME-ENCAPSULATED ANTI-VIRAL COMPOSITION AND METHOD

(57) Abstract

A method and composition for treating viral infections by liposome-encapsulation of the anti-viral compounds phosphonoformate and phosponoacetate. The concentration of anti-viral compound within the liposomes can be adjusted to produce a significant enhancement of anti-viral activity, with minimum increase in cell toxicity.

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LIPOSOME-ENCAPSULATED ANTI-VIRAL COMPOSITION AND METHOD

1. Field of the Invention

The present invention relates to therapeutic compositions and methods for treating viral infections, and more particularly, for enhancing the efficacy of phosphonoacetate and phosphonoformate by liposome encapsulation.

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3. Background of the Invention

Phosphonoformate (PF) and phosphonoacetate (PA) have been shown to be effective inhibitors of viral replication for a variety of DNA (PF and PA) (Helgstand; Oberg; Overby, 1974, 1977), and RNA (PF) containing viruses (Oberg; Sandstrom; Sundquist). These compounds are potent inhibitors of cell-free DNA polymerases (Oberg; Overby, 1977) and reverse transcriptase (Sandstrom; Sundquist). However, greater than 100 fold more drug is necessary to obtain antiviral effects in tissue culture and animals (Oberg; Overby, 1977).

The reason for the decreased activity in intact cells is due, at least in part, to the low cell permeability of the phosphono-compounds. The pK_a's of the three titratable group of PF are 7.3, 3.4 and 0.5 (Warren) while the analogous values for PA are 8.2, 5.0 and 2.6 (Mao). The poor penetration can be attributed to the multianionic nature of the phosphono-compounds at physiological pH. Attempts to enhance their transport properties by chemical modifications have failed to produce more active analogs, even though a large number of congeners have been synthesized and tested (Eriksson; Herrin; Mao).

Liposome delivery systems have been proposed for a variety of drugs. When used for drug delivery via the bloodstream (parenteral drug administration), liposomes have the potential of providing a controlled, "depot" release of liposome-entrapped drug over an extended time period, and of reducing toxic side effects of the drug, by limiting the concentration of free drug in the bloodstream. Liposome/drug compositions can also increase the convenience of therapy by allowing higher drug dosage and less frequent drug administration. In addition, liposomes have been shown to facilitate drug uptake for a small number of drugs (Heath, 1983, 1985).

4. Summary of the Invention

One object of the present invention is to provide a composition and method which enhance the anti-viral activity of PF and PA.

More particularly, it is an object of the invention to enhance the anti-viral activity of the compounds severalfold, without increasing cellular toxicity significantly.

A more specific object of the invention is to provide an improved composition and method for treating

Simplex Herpes, Virus, or (HSV-2) and Human Immunodeficiency Virus (HIV).

The invention includes, in one aspect, a liposome composition composed of an anti-viral agent selected from the group consisting of phosphonoacetate (PA) and phosphonoformate (PF) encapsulated in liposomes. The liposomes are preferably negatively charged, to minimize problem of liposome agglutination on storage and in vivo, and also preferably have a homogeneous size distribution, in a size range less than about 0.4 micron, to allow filter sterilization.

The invention further includes a suspension of liposomes containing PF or PA predominantly in liposome-encapsulated form. The concentration of anti-viral compound which is encapsulated in the liposomes is adjusted to achieve a therapeutic ratio which is substantially greater than that achievable by parenteral administration of the anti-viral compound in non-encapsulated form. The suspension is used to enhance the therapeutic effectiveness of the anti-viral compounds, by increased cellular uptake of virus-infected mamallian cells. This aspect of the invention is based on the discovery that PF and PA show severalfold higher intracellular anti-viral activity when administered in liposome-encapsulated form. According to another aspect of the discovery, the ratio anti-viral activity to cell toxicity can be maximized by adjusting the concentration of drug within the liposomes. This feature takes advantage of the saturable nature of liposome uptake by the cells, as will be seen.

Further included in the invention is a method of facilitating the uptake of PA or PF into virus-infected mammalian cells, and a method of treating an individual for an infection of a virus which is

responsive to either or both of PA or PF. The latter method involves administering a liposome suspension of the type described above to the individual in a therapeutically effective amount. As above, the liposomes are preferably negatively charged and have substantially uniform sizes in a selected size range less than about 0.4 microns. Exemplary viral infections against which the method may be used are HSV-2 and HIV.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figure 1 illustrates the concept of a liposome-dependent drug exemplified by PF and the modes of entry of the drug into a cell;

Figures 2A and 2B compare the antiviral effect in HSV-2 infected VERO cells of free drug (closed squares), free drug and empty liposomes (open circles), and liposome-encapsulated drug (open squares) for PA (A) and PF (B);

Figures 3A and 3B show the inhibition of thymidine incorporation in VERO cells by free drug (closed squares) and liposome-encapsulated drug (open squares) for PA (A) and PF (B);

Figures 4A and 4B show liposome uptake of ^{125}I-labelled liposomes in culture as a function of time after liposome addition (A) and as a function of lipid concentration (B); and

Figure 5 compares the uptake of PF (open squares) and PA (closed squares) by VERO cells after delivery in liposomes, where the dashed line originating at the origin and proceeding through the points marked B, C and E is the curve relating extracellular free drug concentration to intracellular drug concentration.

Detailed Description of the Invention

Materials, Formulations and Drug Effect Studies

Phosphonoacetic acid (PA) and cholesterol were obtained from Sigma Chemical Co., St. Louis, Mo. Egg phosphatidylcholine, egg phosphatidylglycerol were purchased from Avanti Polar Lipids, Birminham, Ala. The p-hydroxybenzamidine dihexadecylphosphatidylethanolamine was synthesized and iodinated as described (Abra). ^{3}H-thymidine was a product of Amersham, Arlington Heights, Il.

Drug and Liposome Preparation: Drug solutions were prepared at 80 mM. The pH was adjusted to 7.4 using either HCl or NaOH and the osmolality was adjusted to 300 mOsm with NaCl before encapsulation. Reverse phase evaporation liposomes (REV) composed of egg phosphatidylcholine /egg phosphatidylglycerol/cholesterol in a molar ratio of 9/1/8 were prepared and extruded through a 0.2 m polycarbonate membrane as described (Szoka, 1978). Nonencapsulated drugs were removed by dialysis against 2 x 100-fold excess of 5 mM Hepes-140 mM NaCl-0.1 nM diethylenetriaminepentaacetic acid pH 7.4 buffer. Non-drug containing (NDC) liposomes were prepared by encapsulating the Hepes buffer. The concentrations of encapsulated drugs and phospholipids were determined by assaying the phosphate content after acid digestion by

the method of Bartlett. The vesicle sample was etracted in a two phase system by the method of Bligh and Dyer. The methanolic aqueous phase contained the drug. The chloroform phase contained the phospholipid. Liposome diameter was determined by a Coulter NS-4 laser light scattering apparatus.

Virus and Cell Culture: Herpes simplex virus 2 (HSV-2), strain G, and VERO cells were obtained from Dr. D. Eppstein, Palo Alto, Ca. VERO cells were routinely grown in Dulbecco's modified essential medium supplemented with 5% heat-inactivated fetal calf serum obtained from the UCSF cell cuture facility. The virus was propagted in VERO cells and titers of various viral preparations were determined by plaque assay in VERO cells (Mogensen).

Cytopathic Effect Assay (CPE): A modified version of the cytopathic effect assay developed by Epstein et al. (Epstein, 1981) was performed as follows. One x 10^4 VERO cells were cultured in a 96-well plate for 2 days at 37°C. After removal of culture fluid, cells were infected by absorbing HSV-2 (800 plaque forming units (PFU)/well) to cells at 37°C for one hour before drug treatment. Various concentrations of drugs, liposomal drugs and drugs plus NDC liposomes (NDC-REV) were added to each well. Each dose was tested in quadruplicate. Controls included in each plate comprised a cell control and a virus control. After 48 hours, cells were rinsed with phosphate buffered saline and then fixed and stained with Armstrong's solution (0.5% W/V crystal violet, 50% ethanol (V/V), 5% formalin (V/V) and normal saline (Epstein, 1981). Excess dye was washed off and the dye incorporated by the viable cells was diluted with dimethyl sulfoxide. The optical densities (OD)

were read at 550 nm using a multichannel spectrophotometer (Titertek-Multiskan). The percentage of dye uptake was calculated as ($OD_{treatment}$ - $OD_{virus\ control}$)/($OD_{cell\ control}$ - $OD_{virus\ control}$) x 100. The ED_{50} is the concentration of the drug at which the test well demonstrates 50% of the control well dye uptake.

Virus Yield Assay: About 10^6 VERO cells cultured in a 6-well (35 mm diameter) plate were inoculated with HSV-2 with a multiplictiy of infection (MOI) of 0.05. Drug treatment started one hour after virus adsorption. Two days later, cell suspensions were subjected to 3 cycles of freezing and thawing to release virus. Virus in the supernatant of the cell cultures were determined by plaque assay in VERO cells with a 1.5% of sea plaque agarose (Marine Colloid Bio-products, FMC, Rockland, Me.) overlay. After the virus plaques had developed (2 days), cells were stained with a 0.01% neutral red solution (Epstein, 1983).

Cytoxicity Assay: VERO cells, without virus infection, were exposed to drug treatment for 44 hours at the same culture conditions as described for the CPE assay. Then, 1 mCi of ^{3}H-thymidine was added to each well for 6 hours. At the end of pulse, cells were lysed and collected onto glass-fiber filters with a multiple channel cell harvester (Skatron Inc., Sterling, Va.). Radioactivity (CPM) associated with each filter disc was counted in a Beckman liquid scintillation spectrometer. Percentage of ^{3}H-thymidine incorporation was expressed as ($CPM_{treatment}/CPM_{control}$) x 100. The IC_{50} is the concentration of the drug required to reduce thymidine incorporation to 50% of the control value.

Uptake of Liposomes by VERO cells: Trace amount of ^{125}I-p-hydroxybenzamidine dihexadecylphosphatidylethanolamine was incorporated in liposomes as a marker for uptake studies as described previously (Abra). VERO cells (1.2×10^6) in 35 mm culture dishes were incubated with various amounts of ^{125}I-labeled liposomes. After incubation for the times indicated, non-attached liposomes were removed by rinsing the cultures 3 times with phosphate buffered saline and the cells were then lysed with 0.5 N NaOH. Radioactivities asociated with the cell lysate were measured in a Beckman gamma scintillation spectrometer. The concentrations of cellular protein in the lysate were determined by the method of Lowry et al.

Results

Liposome Preparations

PF and PA are highly water soluble compounds that can be readily encapsulated in liposomes at high concentrations. Liposome encapsulation resulted in 0.20 mmoles PF/mmole lipid and 0.31 mmoles PA/mmole lipid. Due to the negative charges on the phosphonocompounds at pH 7.4, there is little leakage (less than 1% per week) of either PF or PA from the liposomes upon storage at 4°C. The diameter of the liposomes as measured by dynamic light scattering ranged between 0.16-0.22 m for various preparations.

Antiviral Effects

To measure the antiviral efficacy of the liposome encapsulated compounds a quantitative dye binding cytopathic effect assay was utilized (Epstein, 1983). In this assay non-encapsulated PA had an ED_{50} of about 60 mM, and the addition of non-drug containing

(NDC) liposomes did not change this value (Fig. 2A, Table 1). Liposome encapsulation of PA resulted in a 150 fold increase in efficacy (ED_{50} = 0.4 mM) (Fig. 2A, Table 1). Non-encapsulated PF in the presence or absence of NDC liposomes had an ED_{50} of about 210 mM. Encapsulation of PF resulted in about a 30 fold increase in efficacy, ED_{50} = 7 mM (Fig. 2B, Table 1). Virus yield assays confirmed the increased effectiveness of the liposome encapsulated PF (Table 2). In the virus yield assay about a 10 fold increase in efficacy was seen with the liposome encapsulated drug compared to the non-encapsulated PF.

Comparison of the efficacy and toxicity of liposome encapsulated PF and PA with the non-encapsu lated compounds:

TABLE 1

Agent	ED_{50}		IC_{50}		Selectivity	Enhancement
PA	60±25	(2)	507±162	(3)	8.4	
Lipo PA	0.40±0.1	(2)	2.1±84	(4)	5.25	0.63
PF	210±84	(4)	880±57	(5)	4.2	
Lipo PF	7±2.9	(4)	800±(5)	(4)	114	27

ED_{50} = the concentration (mM) % S.D. required to reduce the cytopathic effect to 50% of the untreated viral controls as described in the Methods. The value in parentheses are the number of independent determinations.

IC_{50} = the concentration (mM) % S.D. required to reduce thymidine incorporation in VERO cells to 50% of the control value as described in the Methods. The value in parenthesis is the number of independent determinations.

Selectivity = the ratio of the IC^{50} to the ED_{50}.

Enhancement = the ratio of the selectivity of the liposomal PF to the selectivity of the non-encapsulated PF.

TABLE 2

Comparison of virus yield following treatment with PF or liposomal PF. VERO cells were infected with HSV-2 virus and treated with drugs for 2 days. Virus titer of the supernatant from the cell lysate were determined using the paque assay on VERO cells in quadruplicate as described in the Methods.

Treatment	Concentration	Virus yield[a] (x 10^6 PFU/ml)
Control	20 mM	7.38 % 1.61 (100.0 %)
Free PF	100 mM	8.52 % 0.01 (115.4 %)
	500 mM	2.38 % 0.22 (32.2 %)
Liposomal PF	2 mM	6.74 % 1.42 (91.3 %)
	10 mM	4.04 % 0.69 (54.7 %)
	50 mM	2.22 % 0.35 (30.0 %)

[a]Mean % standard deviation (S.D.)

Inhibition of Thymidine Incorporation

The inhibition of incorporation of thymidine intraellular DNA was used as a measure of the cytotoxicity of the drugs. Thymidine incorporation assays were performed using the same tissue culture conditions as for the CPE assay. PA had an IC_{50} of 507 mM which was reduced considerably to 2 mM when the drug was encapsulated in liposomes (Fig. 3A, Table 1). In contrast, PF exhibited an IC_{50} of 900 mM which was not appreciably reduced by liposome encapsulation (Fig. 3B, Table 1).

Although PA in liposomes was approximately 150 fold more active as an antiviral, it was also 250 fold more cytotoxic. Therefore, its selectivity ratio (ED_{50}/IC_{50} = 5.3) was less that that for the free drug (Table 1). Encapsulation of PF in liposome resulted in a 30 fold increase in antiviral activity but no increase in cytotoxicity. Thus, the selectivity ratio of the PF was increased from 20 to approximately 100 by liposome encapsulation. This unexpected increase in the selectivity ratio for liposomal PF was replicated in five separate experiments.

Liposome Uptake by VERO Cells

A possible explanation for the increase in the selectivity ratio for the liposomal PF but not for liposomal PA is that liposome uptake by the VERO cells is saturable. The maximum amount of lipsome encapsulated drug that becomes cell associated is a function both of the saturation level of cell-associated liposomes as well as the concentration of the drug in the liposome. When the drug does not leak from the lipsome until it is internalized by the cell, intracellular drug concentrations that inhibit viral

replication but not cellular DNA sysnthesis can be attained.

Measurement of the cellular association of a nonexchangeable radiolabeled lipid marker demonstrated that liposome uptake plateaued after 24 hours (Fig. 4A). Moreover, the uptake saturated at a liposome dose of about 300 mM (Fig. 4B). If the liposomes retain the encapsulated drug during the cellular uptake process, the amount of drug that becomes cell associated is the product of the amount of drug per liposome times the number of liposomes that are cell associated (Fig. 5). The difference in the computed cell associated drug between PF and PA is due to a higher encapsulation ratio for PA. For comparison, uptake of non-encapsulated PF is assumed to be a linear function of the external drug concentration (Fig. 5). It is evident that a majority of drug uptake from the liposome form, occurs at low levels of added liposome-encapsulated drug. The consequence of this for drug action will be discussed in the next section.

Design of Optimal Formulations

The order of magnitude increases in anti-herpes simplex 2 efficacy achieved by the encapsulation of PF or PA in liposome dependent drug is important for antiviral therapy in vivo using these compounds.

In the case of PF the increased efficacy was achieved without any substantial increase in cytotoxicity as measured by inhibition of thymidine incorporation. Thus, the selectivity ratio of liposomal PF was significantly better than the non-encapsulated compound. PA, on the other hand, exhibited a 150 fold increase in efficacy but also had a 250 fold increase in cytotoxicity so that the selectivity of the drug was

slightly decreased. We postulate that the difference between the two phosphonocompounds relates to 3 factors: (1) the difference in the encapsulation ratio, that is, the mmole of drug to mmole of lipid, achieved in the preparation of the liposomes, (2) the relative sensitivity of the VERO cell to the cytotoxic effects of the two drugs, and (3) the fact that liposome uptake by the VERO cells saturates (Figs. 4, 5).

In Figure 5 this concept is illustrated graphically. It is assumed that drug uptake for the encapsulated compound is directly related to liposome uptake, and that no leakage of the drug from the liposome or liposome-cell complex into the medium occurs. Another assumption is that uptake of the non-encapsulated drug is linearly proportional to drug in the culture medium. Finally, it is assumed that the antiviral effect and the cytotoxic effect occurs at the same intracellular drug concentration, regardless of whether the drug is delivered in liposomes or as the non-encapsulated compound.

In the case of PF, the drug concentration delivered in liposomes that inhibits viral replication (ED_{50}) can be estimated from the liposome uptake data, since the fraction of the liposomes that become cell associated at any lipid concentration is known. This inhibitory livel of PF can be achieved intracellularly by the non-encapsulated drug, albeit at a higher external concentration (Fig. 5). The assumption of a linear relationship between external and internal drug levels allows construction of the dashed line in Figure 5 which passes through the origin and point B (the inhibitory drug level for non-encapsulated compound at ED_{50} = 200 mM). Given that the same intracellular drug concentration would exist at ED_{50} for liposome encapsulated or non-encapsulated compound, point B can

be obtained from knowing the inhibitory drug level at ED_{50} for the liposome encapsulated drug (point A). At the IC_{50} (900 mM) of free PF, the intracellular concentration (point C) exceeds that attainable by the liposomal form (point D). Thus, the liposome can increase the efficacy without increasing cytotoxicity of the encapsulated PF. If the same dashed line (Fig. 5) is used to represent the intracellular concentration attained by the non-encapsulated PA, then the extracellular concentration of PA that caused cytotoxicity (point E) results in an intracellular concentration of PA that can be achieved by a low level of liposomal PA (point F). Thus, both the efficacy and toicity would be increased for PA when it is encapsulated.

Based on this model, a decrease of 5 fold in the PA concentration encapsulated in liposomes, should still result in an enhancement of the antiviral effect but should limit the maximal concentration of PA in the cell to below the cytotoxic range. When this was done, the efficacy increased 40 fold while the cytotoxicity was not changed.

The optimal use of liposomes dependent drugs <u>in vivo</u> will depend upon targeting of the liposome. This can be done either by an active scheme when a targeting ligand is employed or by exploiting the known capacity of macrophages to take up liposomes (Szoka, 1986). If the latter approach is taken, viral diseases that infect the macrophage would be ideal targets for such therapy. Clearly, PF or PA appear to be good candidates for liposome encampsulation for therapy of virally infected macrophages.

The antiviral potency of the phosphono-compounds is hindered by their inability to cross membranes, nonetheless, phosphonoformate has been

used to treat cytomegalovirus infections in bone marrow and renal transplant recipients (Ringden). Moreover, the potent anti-reverse transcriptase activity of PF (Sandstrom, Sundquist) has already been exploited in a limited clinical trial to treat human immunodeficiency virus (HIV) infections (Farthing, Gaub). In these studies, up to 15 g of PF per day has been infused on a continuous basis, for a period of 21 days. The large dose and continuous IV infusion is necessary because PF is cleared rapidly from the blood and is poorly absorbed when taken orally. A 100 fold enhancement of efficacy would permit liposome encapsulated PF to be given as an IV bolus rather that as a continuous infusion. In addition, HIV is know to infect macrophages (Crowe; Gartner; Levy; Lifson; Ruscetti), and the macrophage has been suggested by some to be a significant factor in the persistence of the virus and its transport into the brain in vitro (Crowe; Popovic; Sodroski). The liposome effect demonstrated here is not virus specific, and other viral infections sensitive to the phosphono drugs in endocytotically active cells would respond in a similar fashion to the liposome-encapsulated drugs. This feature of the present invention is illustrated by the following treatment method involving Rausher Murine Leukemia Virus (RMLV):

RMLV causes an initial proliferation of erythroblast in the bone marrow and the spleen of infected mice. Splenomegaly, hepatomegaly and viremia ensue. A spleen weight enlargement assay was used to determine the efficacy of the antiviral agent-phosphonoformate in this in vivo model. Specifically, mice were inoculated with diluted virus solution by i.v. injection into the tail vein. Various drug treatment regimens were started the day after. Then mice were sacrificed at 20 days after virus infection. The

resected spleens and livers were weighed. The weights of spleens and livers of each group were compared by using a paired Student's t test.

Protocol

Female mice (6 weeks old) were infected with 0.25 ml of diluted (1:20) RMLV solution by i.v. injection into the tail vein at day 0. Control group received PBS. Drug treatment started at day 1. PF (500 mg PF/kg body weight) was given by i.p. injection for 3 consecutive days and then every other day for 7 doses. For control and virus control groups, 0.3 ml of PBS were given by i.p. injection at the same schedule. Liposomal PF (20 mg/kg) was administered by i.v. injection for 3 consecutive days and then given by i.p. injection every other day for 7 doses. At day 20, all the mice were sacrificed and their spleens and livers were weighed.

Results

Spleen weight and liver weight of tested mice were compared respectively among groups with various treatments. There were significant differences (pF0.01) in spleen weight between the drug treatment groups and the virus control group. Mice treated with liposomal PF or nonencapsulated drug had similar spleen weights even though the nonencapsulated drug dosage was 25 times greater than liposomal drug dosage. This increased efficacy of liposomal PF was comparable with results obtained from an in vitro study using Herpes simplex 2 virus model. In terms of alleviating hepatomegaly caused by RMLV, non-encapsulated PF seemed to be ineffective. Meanwhile, liposomal PF treatment resulted in a reduced liver weight when compared with the virus control group (pF0.02). These results surely suggest that encapsulation of PF in liposomes causes an

enhanced efficacy in antiviral therapy both in vivo and in vitro.

While preferred liposome compositions and drug-treatment methods have been described, it will be appreciated that alternative compositions, methods of preparations and methods of are within the scope of the invention, as described and claimed herein.

IT IS CLAIMED:

1. A liposome composition comprising an anti-viral compound selected from the group consisting of phosphonoacetate and phosphonoformate encapsulated in liposomes.

2. The composition of claim 1, wherein the liposomes are negatively charged.

3. The composition of claim 1, wherein the liposomes have substantially uniform sizes in a selected size range less than about 0.4 microns.

4. The composition of claim 1, wherein the anti-viral compound is phosphonoformate.

5. A suspension of liposomes containing an anti-viral compound selected from the group consisting of phosphonoacetate and phosphonoformate predominantly in liposome-encapsulated form.

6. The suspension of claim 5, for use in treating a viral infection, by parenteral administration of the suspension, wherein the liposomes are negatively charged and have substantially uniform sizes in a selected size range less than about 0.4 microns.

7. The suspension of claim 6, wherein the concentration of anti-viral compound which is encapsulated in the liposomes is adjusted to achieve a therapeutic ratio which is substantially greater than that achievable by parenteral administration of the anti-viral compound in non-encapsulated form.

8. The suspension of claim 7, wherein the anti-viral compound is phosphonoformate.

9. A method of facilitating the uptake of an anti-viral compound selected from the group consisting of phosphonoacetate and phosphonoformate by mamallian cells comprising providing a suspension of liposomes containing the anti-viral compound predominantly in liposome-encapsulated form, and contacting the cells with said liposomes.

10. A method of treating an individual for an infection of a virus which is responsive to an anti-viral compound selected from the group consisting of phosphonoacetate and phosphonoformate comprising

providing a suspension of liposomes containing the anti-viral compound predominantly in liposome-encapsulated form, and

administering the the suspension parenterally to the individual in a therapeutically effective amount.

11. The method of claim 10, wherein the liposomes are negatively charged and have substantially uniform sizes in a selected size range less than about 0.4 microns.

12. The method of claim 10, wherein the anti-viral compound is phosphonoformate.

13. The method of claim 12, wherein said providing includes adjusting the concentration of the anti-viral compound in the liposomes to achieve a therapeutic ratio which is substantially greater than

that achievable by parenteral administration of the anti-viral compound in non-encapsulated form.

14. The method of claim 10, for use in treating an infection by Herpes Simplex Virus-2.

15. The method of claim 10, for use in treating infection by a Human Immunodefficiency Virus.

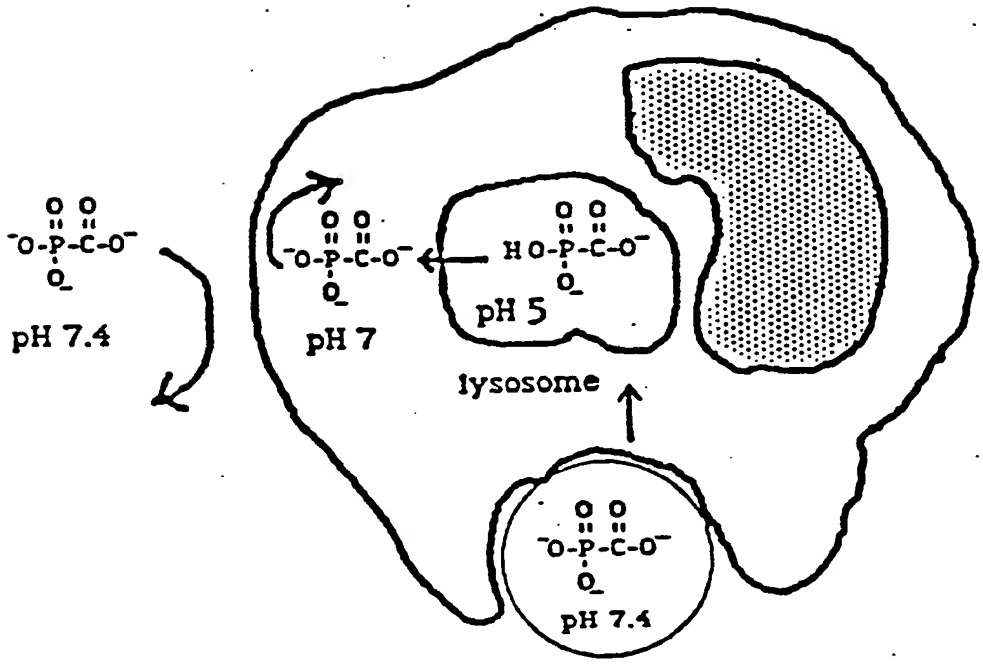

Figure 1

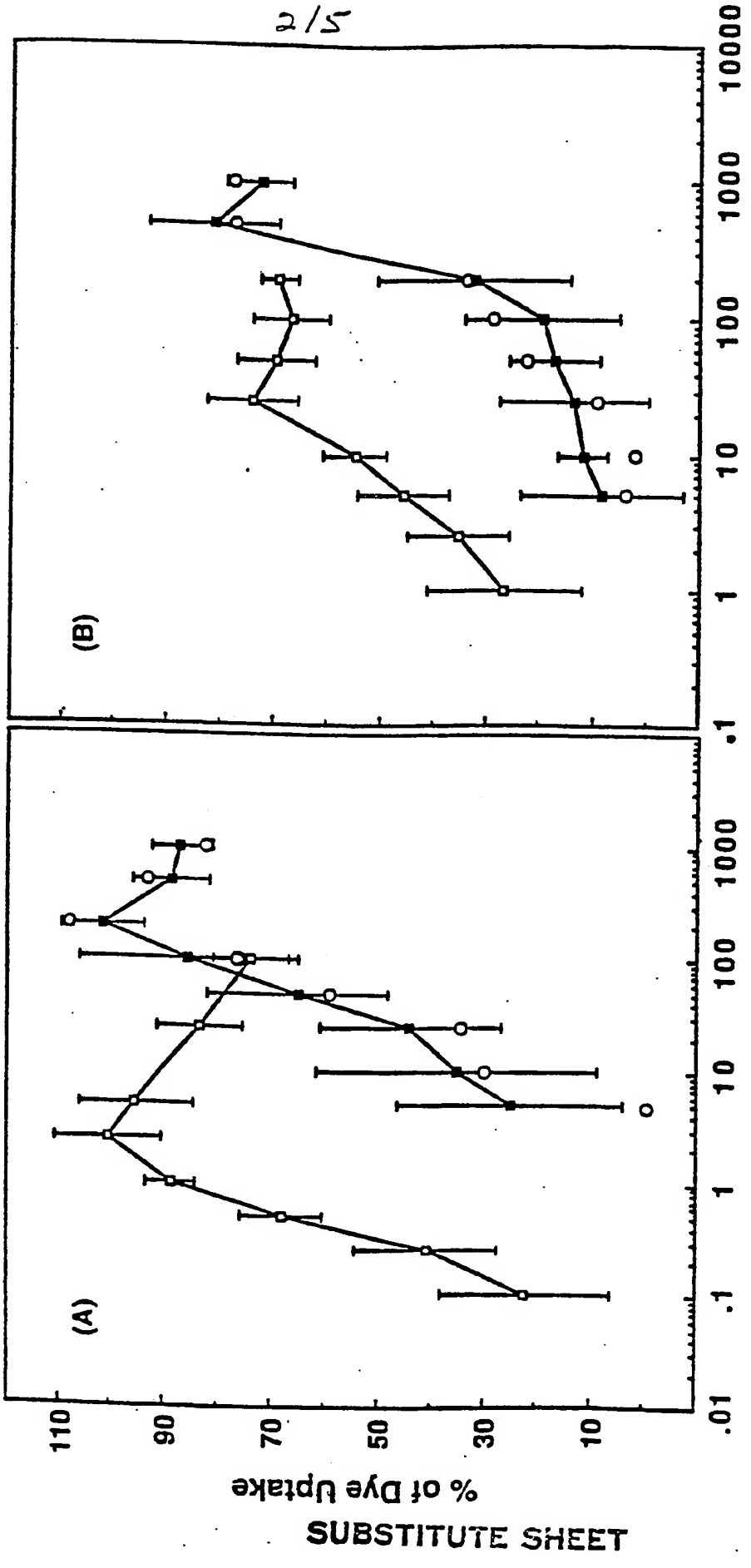

Figure 2A

Figure 2B

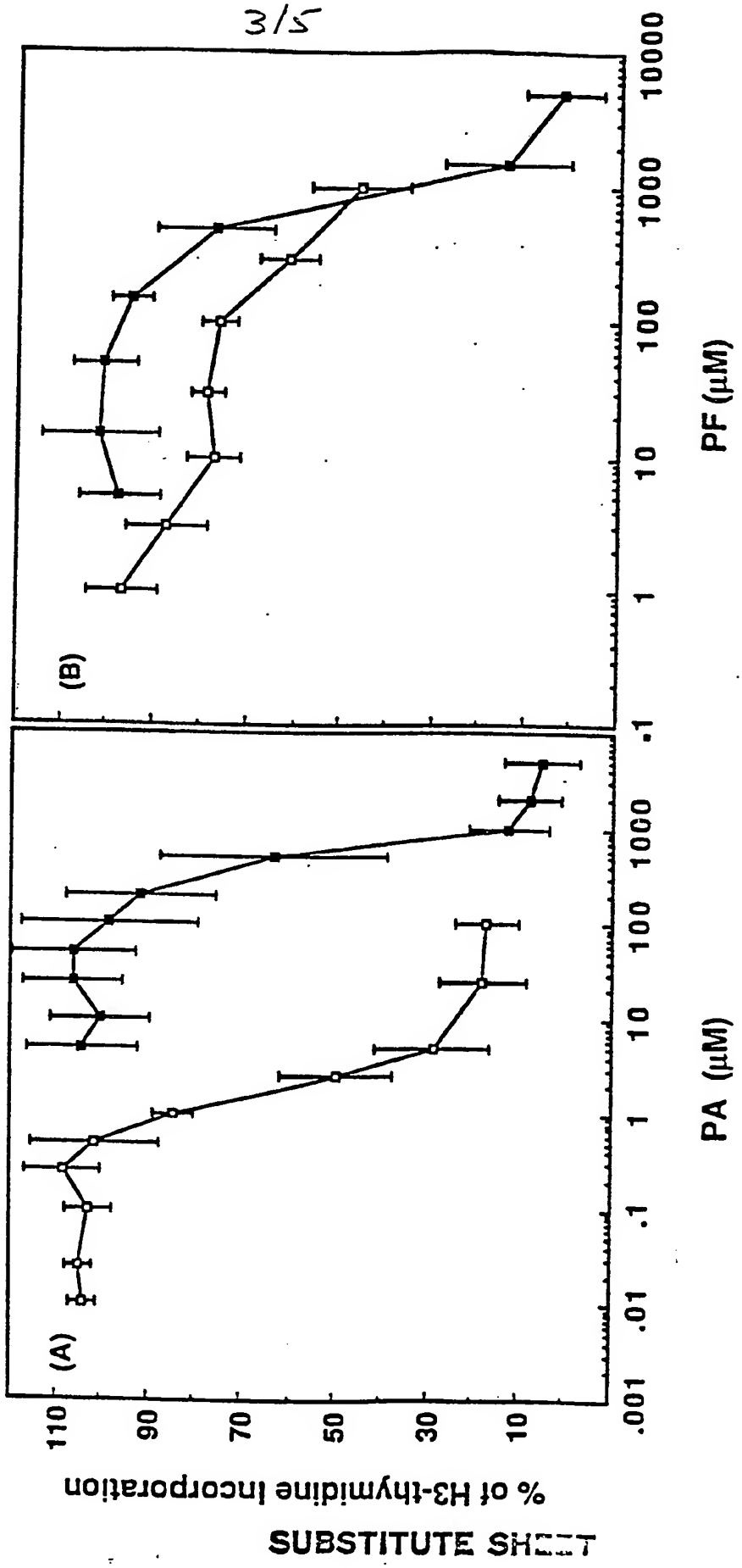

Figure 3A

Figure 3B

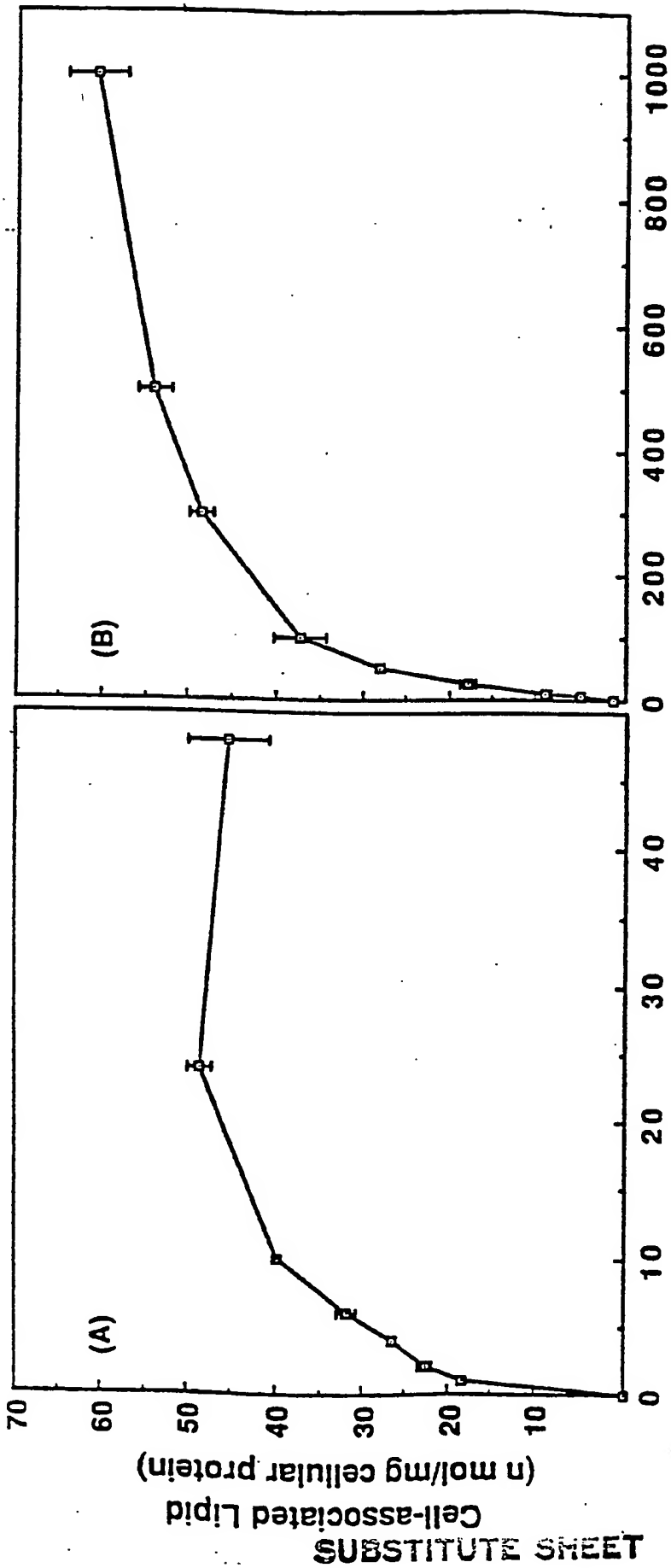
Figure 4A
Figure 4B
(A)
(B)
Cell-associated Lipid
(n mol/mg cellular protein)
Time (Hours)
Lipid Concentration (μM)
0
10
20
30
40
50
60
70
200
400
600
800
1000

5/5

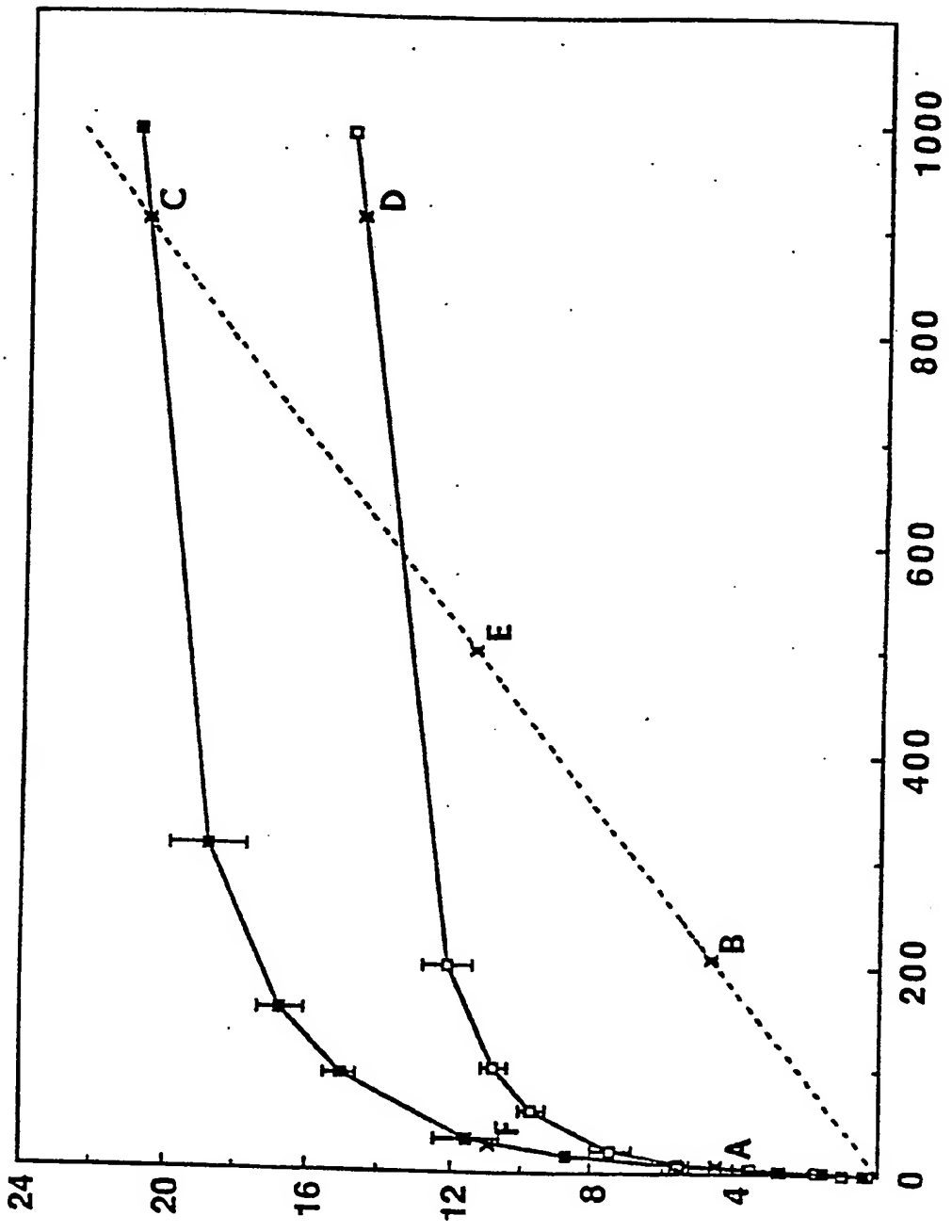
Cell-asso. Amount
(nmol/mg cellular protein)
24
20
16
12
8
4
0
200
400
600
800
1000
Drug Concentration (μM)
A
B
C
D
E
F

Figure 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/US88/004333

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4) A61K 37/22, 9/48

U.S. CL. 424/450; 424/451

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S. Cl.	424/450; 424/451

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 4,663,161 (MANNINO, ET AL) 05 MAY 1987. SEE ENTIRE DOCUMENT.	1-15
Y	US, A, 4,724,232 (RIDEOUT ET AL) 09 FEBRUARY 1988. SEE ENTIRE DOCUMENT.	1-15
Y	US, A, 4,752,425 (MARTIN, ET AL) 21 JUNE 1988. SEE ENTIRE DOCUMENT.	1-15

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
10 JANUARY 1989	03 APR 1989
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	PENNY L. PRATER

Form PCT/ISA/210 (second sheet) (October 1981)